



Short communication

Branched-chain C-cyano pyranonucleosides: Synthesis of 3'-C-cyano & 3'-C-cyano-3'-deoxy pyrimidine pyranonucleosides as novel cytotoxic agents

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ABSTRACT

This report describes the total and facile synthesis of 3'-C-cyano & 3'-C-cyano-3'-deoxy pyrimidine pyranonucleosides. Reaction of 3-keto glucoside **1** with sodium cyanide gave the desired precursor 3-C-cyano-1,2:5,6-di-*O*-isopropylidene- α -D-glucopyranose (**2**). Hydrolysis followed by acetylation led to the 1,2,3,4,6-penta-*O*-acetyl-3-C-cyano-D-glucopyranose (**4**). Compound **4** was condensed with silylated 5-fluorouracil, uracil, thymine and *N*⁴-benzoylcytosine, respectively and deacetylated to afford the target 1-(3'-C-cyano- β -D-glucopyranosyl)nucleosides **6a–d**. Routine deoxygenation at position 3' of cyanohydrin **2**, followed by hydrolysis and acetylation led to the 3-C-cyano-3-deoxy-1,2,4,6-tetra-*O*-acetyl-D-allopyranose (**10**). Coupling of sugar **10** with silylated pyrimidines and subsequent deacetylation yielded the target 1-(3'-C-cyano-3'-deoxy- β -D-allopyranosyl)nucleosides **12a–d**. The new analogues were evaluated for their antiviral and cytostatic activities. It was found that **6a** was endowed with a pronounced anti-proliferative activity that was only 2- to 8-fold less potent than that shown for the parental base 5-fluorouracil. None of the compounds showed activity against a broad panel of DNA and RNA viruses.

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1. Introduction

Modified nucleosides constitute a major class of biologically active compounds, especially as antitumor and antiviral agents [1–4]. Cytotoxic nucleoside analogues were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer [5]. Nucleoside chemistry has also evolved to facilitate efficient routes to effective agents for the treatment of viral diseases caused by HIV [6] and herpes viruses [7]. Subsequently, nucleosides, which are frequently altered in the carbohydrate or base moiety, became the focus for the development of novel chemotherapeutic agents.

During the last decades, several branched-chain sugar nucleosides have been extensively studied for their potential antitumor or antiviral properties [8–11]. Attachment of the cyano group to the sugar moiety has been an attractive object for nucleoside chemists due to its small size and its great electron withdrawing character. Thus, cyano ribofuranose nucleosides have been reported as interesting antiviral agents [12–14], while replacement of the hydroxyl group of 1- β -D-arabinofuranosylcytosine (ara-C) by the

cyano group led to a new biologically active compound [15,16], with a novel mechanism of anticancer action [17].

Lately, nucleosides bearing pyranosyl rings have been evaluated for their potential antiviral [18–20], antioxidant [21] and antibiotic [22] properties and as building blocks in nucleic acid synthesis [23,24]. As part of our efforts to develop novel biologically active agents, we recently reported that new classes of uncommon 3'-fluorinated pyranonucleosides have a promising potential in combating the rotaviral infections and in the treatment of colon cancer, and are efficient as antitumor growth inhibitors [25–29]. Experimental data also revealed that human poly(A)-specific ribonuclease [30] and glycogen phosphorylase [31] are among the molecular targets of these compounds.

In view of the interesting biological activity of the fluorinated pyranonucleosides, we decided to extend our studies to the synthesis of novel molecules in which an electron withdrawing cyano group replaces the fluorine atom. Therefore, we report the stereocontrolled synthesis of novel branched-chain C-cyano pyrimidine pyranonucleosides, i.e. 3'-C-cyano- β -D-glucopyranonucleosides and 3'-C-cyano-3'-deoxy- β -D-allopyranonucleosides, bearing 5-fluorouracil, uracil, thymine and cytosine as heterocyclic bases, in order to assess their biological activity. The chemical synthesis and biological activity of these compounds are presented herein.

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2. Results and discussion

2.1. Chemistry

3'-C-Cyano- β -D-glucopyranonucleosides **6a–d** were prepared according to the synthetic route outlined in Scheme 1. Treatment of the 1,2:5,6-di-O-isopropylidene- α -D-ribo-hexofuranos-3-ulose (**1**) [32] with sodium cyanide in a two-phase ethylether/H₂O system, in the presence of sodium bicarbonate, afforded the thermodynamically more stable gluco cyanohydrin epimer **2**, in a virtually quantitative yield [33,34]. The assignment of its configuration was further supported by NOE measurements, as depicted in Fig. 1. A 5% NOE enhancement of H-2 and a 7% NOE enhancement of H-5 on irradiation of the proton of the free OH group show that these protons are on the same side of the ring system. Hydrolysis of **2** using Amberlite IR 120 (H⁺) resin in methanol followed by acetylation using acetic anhydride (Ac₂O) in pyridine [32] led to the 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-glucopyranose (**4**). The protected, 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano- β -D-glucopyranosyl) pyrimidine nucleosides **5a–d** were obtained upon coupling of the precursor material **4** with silylated 5-fluorouracil, uracil, thymine and N⁴-benzoylcytosine, respectively, in the presence of trimethylsilyl trifluomethane-sulfonate (Me₃SiOSO₂CF₃), in refluxing acetonitrile [35]. The ¹H NMR spectra obtained for the protected nucleosides **5a–d**, showed large coupling constants between protons H-1' and H-2' ($J_{1',2'} = 9.4$ – 9.6 Hz), indicating an axial orientation of both protons and equatorially oriented base rings. Fully deprotection of **5a–d**, performed by saturated methanolic ammonia [36], gave the desired nucleosides **6a–d**.

Compound **2** was the starting material for the synthesis of 3'-C-cyano- β -D-allopyranonucleosides **12a–d** (Scheme 1). Phenox-ythiocarbonylation of **2** under a commonly used condition, phenyl chlorothionoformate, 4-(dimethylamino)pyridine (DMAP) and triethylamine (Et₃N) in CH₃CN [16], afforded the 3'-O-phenox-ythiocarbonyl derivative **7**, which proved to be unstable during the purification process. Therefore, crude **7** was directly submitted to deoxygenation with Bu₃SnH in the presence of 2,2'-azobis(isobutyronitrile) (AIBN), to give the 3'-deoxy derivative sugar **8**, in 76% overall yield. In order to elucidate the structure of the newly synthesized **8**, NOE measurements were performed, as depicted in Fig. 1. The mutual NOE enhancements observed between H-2 with both H-1 and H-3 show that all these protons are in the same β face of the furanose ring. In this type of radical deoxygenation, the hydrogen atom enters from the less hindered, β -face of the planar radical intermediate, opposite to the bulky 1,2-O-isopropylidene group.

Hydrolysis of **8** using Amberlite IR 120 (H⁺) resin in methanol followed by direct standard acetylation led to the 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranose (**10**). Condensation of cyano sugar **10** with per-O-silylated 5-fluorouracil, uracil, thymine and N⁴-benzoylcytosine using Me₃SiOSO₂CF₃ as activator, afforded the 1-(2',4',6'-tri-O-acetyl-3'-C-cyano-3'-deoxy- β -D-allopyranosyl) pyrimidine nucleosides **11a–d**, respectively [35]. ¹H NMR data obtained for the newly synthesized nucleosides **11a–d** ($J_{1',2'} = 9.4$ – 9.7 Hz, $J_{2',3'} = 5.0$ – 5.2 Hz), revealed the β -configuration of the sugar moiety and an axial oriented cyano group, respectively.

Finally, treatment of **11a–d** with hydrazine hydrate in buffered acetic acid (AcOH)–pyridine gave the fully deprotected nucleosides **12a–d**, in yields that varied from 55% to 75%. Interestingly, attempts to deprotect **11a–d** by NH₃/methanol (MeOH) under the subsequent basic conditions produced complex mixtures, containing β -elimination products, probably due to the acidity of H-3' and the presence of two leaving groups (OAc) at the 2'- and 4'-positions of the sugar moiety.

2.2. Biological activity

The cytostatic activity of **6a–d** and **12a–d** was determined against murine leukemia L1210, human lymphocyte CEM and human cervix carcinoma HeLa cell cultures. The test compounds showed poor, if any cytostatic activity against the three cell lines, except the 3'-C-cyano-5-fluorouracil pyranonucleoside **6a** that showed pronounced anti-proliferative activity against all three cell lines. Its cytostatic activity spectrum was similar to that of the parent base 5-fluorouracil. It is currently unclear whether **6a** is biologically active as such, or, alternatively, acts as a prodrug of 5-fluorouracil, from which the free base may be released by the action of phosphorolytic enzymes and/or by a spontaneous release (Table 1).

None of the compounds was endowed with activity against a broad panel of DNA and RNA viruses in cell culture at 100 μ M.

3. Conclusion

In conclusion, the stereocontrolled synthesis of the 3'-C-cyano & 3'-C-cyano-3'-deoxy pyranonucleoside analogues bearing 5-fluorouracil, uracil, thymine and cytosine, respectively was undertaken. The target nucleosides were tested for their inhibitory effects on the proliferation of murine leukemia L1210, human lymphocyte CEM and human cervix carcinoma HeLa cell cultures. 3'-C-Cyano-5-fluorouracil pyranonucleoside **6a** showed a similar cytostatic activity spectrum as the free base 5-fluorouracil.

4. Experimental part

4.1. Chemistry

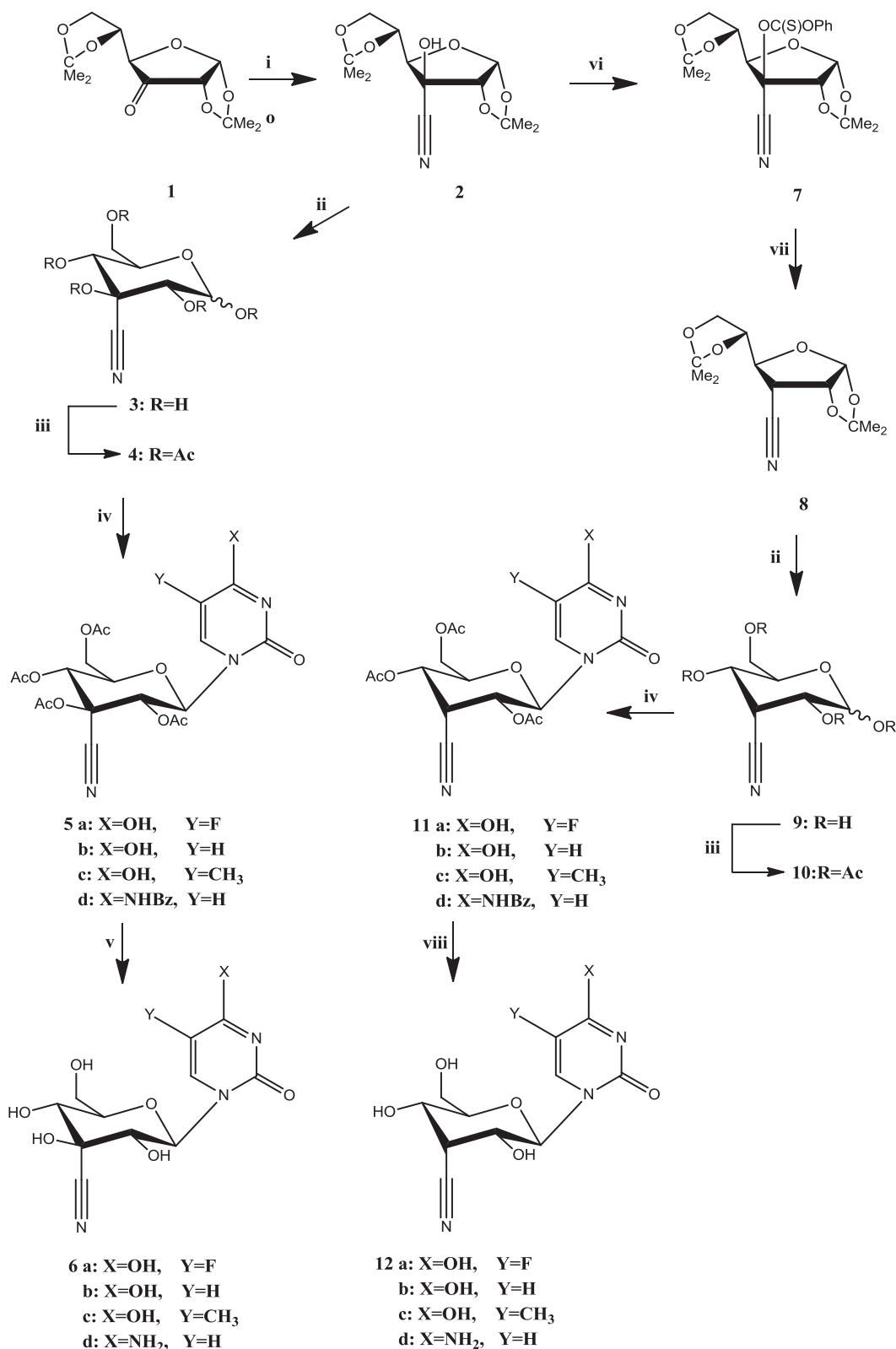
Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Merck precoated 60F₂₅₄ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography was performed using silica gel (240–400 mesh, Merck). ¹H and ¹³C NMR spectra were obtained at room temperature with a Bruker 400 spectrometer at 400 and 100 MHz, respectively, using CDCl₃ and methanol-d₄ (CD₃OD) with internal tetramethylsilane (TMS).

UV–Vis spectra were recorded on a PG T70 UV–VIS spectrometer and mass spectra were obtained with a Micromass Platform LC (ESI-MS). Optical rotations were measured using an Autopol I polarimeter. Infrared spectra were obtained with a Thermo Scientific Nicolet IR100 FT-IR spectrometer. Acetonitrile and toluene were distilled from calcium hydride and stored over 3E molecular sieves.

4.2. Synthesis of 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-glucopyranose (**4**)

4.2.1. Synthesis of 3-C-cyano-1,2:5,6-di-O-isopropylidene- α -D-glucofuranose (**2**)

A mixture of 1,2:5,6-di-O-isopropylidene- α -D-ribo-hexofuranos-3-ulose (**1**) (4 g, 15.5 mmol), H₂O (62 mL), ethylether (124 mL), sodium bicarbonate (2.6 g, 15.5 mmol) and sodium cyanide (0.76 g, 7.75 mmol) was stirred vigorously at room temperature overnight. The organic phase was separated, and the aqueous phase was washed with ethylether (2 x 124 mL). The combined ether phases were dried over Na₂SO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography [(ethylacetate) EtOAc/hexane, 3:7] to give compound **2** (4.28 g, 97%, R_f = 0.40 in EtOAc/hexane, 3:7) as a white solid, mp 98–100 °C [34]. $[\alpha]_D^{22} + 46$ (c 0.3, CHCl₃); ¹H NMR (CDCl₃): δ 5.97 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.59 (d, 1H, H-2), 4.36–4.32 (m, 1H, H-5),



Scheme 1. (i) H₂O, ethylether, NaHCO₃, NaCN; (ii) H₂O, MeOH, Amberlite IR 120 (H⁺); (iii) Ac₂O, pyridine; (iv) Silylated base, CH₃CN, Me₃SiOSO₂CF₃; (v) Methanolic ammonia; (vi) Phenyl chlorothionoformate, Et₃N, DMAP, CH₃CN, 0 °C; (vii) Bu₃SnH, AIBN, toluene, 100 °C; (viii) N₂H₄·H₂O, AcOH, pyridine.

4.24–4.21 (m, 2H, H-6a, H-4), 4.11 (m, 1H, H-6b), 4.04 (s, 1H, 3–OH), 1.58, 1.55, 1.39, 1.37 (4s, 12H, 4CH₃); Anal. Calcd for C₁₃H₁₉NO₆: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.84; H, 6.77; N, 4.82; ESI-MS (*m/z*): 286.32 (M + H⁺).

4.2.2. Synthesis of 3-C-cyano-D-glucopyranose (**3**)

To a solution of **2** (2 g, 7.01 mmol) in MeOH (10.9 mL) and H₂O (62.2 mL) was added Amberlite IR 120 (H⁺) resin and the mixture was refluxed overnight. The reaction mixture was filtered and

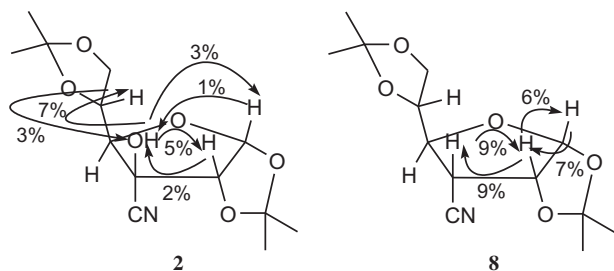


Fig. 1. NOE enhancements measured on compounds **2** and **8**.

evaporated to dryness to give compound **3** (1.25 g, 87%, $R_f = 0.33$ in EtOAc/MeOH, 9:1) as a viscous oil, and it was used without further purification. $[\alpha]_D^{22} - 25$ (c 0.44, MeOH); Anal. Calcd for $C_7H_{11}NO_6$: C, 40.98; H, 5.40; N, 6.83. Found: C, 40.81; H, 5.35, N, 6.92; ESI-MS: (m/z) 206.19 ($M + H^+$).

4.2.3. Synthesis of 1,2,3,4,6-penta-O-acetyl-3-C-cyano-D-glucopyranose (**4**)

Compound **3** (1.25 g, 6.09 mmol) was dissolved in a mixture of pyridine (21.2 mL) and Ac_2O (10.9 mL). The reaction was carried out at room temperature for 1 h, then was quenched with MeOH at 0 °C and was concentrated in vacuum. The residue was diluted with EtOAc, washed with saturated sodium bisulfate, sodium bicarbonate and H_2O . The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give compound **4** (2.28 g, 90%, $R_f = 0.43$ in EtOAc/hexane, 3:7) as a colorless oil. $[\alpha]_D^{22} + 30$ (c 1.30, $CHCl_3$); Anal. Calcd for $C_{17}H_{21}NO_{11}$: C, 49.16; H, 5.10; N, 3.37. Found: C, 49.40; H, 5.12; N, 3.46; ESI-MS: (m/z) 416.36 ($M + H^+$).

4.3. Synthesis of 3'-C-cyano- β -D-glucopyranonucleosides **6a–d**

4.3.1. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano- β -D-glucopyranosyl)5-fluorouracil (**5a**)

A mixture of 5-fluorouracil (109 mg, 0.84 mmol), hexamethyldisilazane (HMDS) (220 μ L, 1.04 mmol) and saccharine (7 mg, 0.039 mmol) in anhydrous CH_3CN (3.5 mL) was refluxed for 30 min under nitrogen. 1,2,3,4,6-Penta-O-acetyl-3-C-cyano-D-glucopyranose (**4**) (0.25 g, 0.6 mmol) and $Me_3SiOSO_2CF_3$ (152 μ L, 0.84 mmol) were then added and the reaction mixture was refluxed for 4 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH_2Cl_2 (200 mL). The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure **5a**

(0.18 g, 60%, $R_f = 0.47$ in EtOAc/hexane, 1:1) as a white solid, mp 114–116 °C. $[\alpha]_D^{22} - 2$ (c 0.37, $CHCl_3$); λ_{max} 262 nm (ϵ 7950); 1H NMR ($CDCl_3$): δ 8.36 (br s, 1H, NH), 7.37 (d, 1H, $J_{6,F5} = 5.6$ Hz, H-6), 6.05 (dd, 1H, $J_{1,2'} = 9.5$ Hz, $J_{1',F5} = 1.2$ Hz, H-1'), 5.70 (d, 1H, H-2'), 5.64 (d, 1H, $J_{4',5'} = 10.2$ Hz, H-4'), 4.45–4.39 (m, 1H, H-5'), 4.22–4.14 (m, 2H, H-6a', H-6b'), 2.16, 2.14, 2.11, 2.04 (4s, 12H, 4OAc); . Anal. Calcd for $C_{19}H_{20}FN_3O_{11}$: C, 47.02; H, 4.15; N, 8.66; Found: C, 47.22; H, 4.21; N, 8.79. ESI-MS (m/z) 486.39 ($M + H^+$).

4.3.2. Synthesis of 1-(3'-C-cyano- β -D-glucopyranosyl)5-fluorouracil (**6a**)

Compound **5a** (0.18 g, 0.37 mmol) was treated with ammonia/MeOH (saturated at 0 °C, 20.6 mL). The solution was stirred overnight at room temperature and then was concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2 /MeOH, 9:1) to afford pure **6a** (76 mg, 65%, $R_f = 0.40$ in CH_2Cl_2 /MeOH, 8:2) as a white foam. $[\alpha]_D^{22} + 20$ (c 0.50, CD_3OD); λ_{max} 266 nm (ϵ 7739); 1H NMR (CD_3OD): δ 7.95 (d, 1H, $J_{6,F5} = 6.7$ Hz, H-6), 6.01 (dd, 1H, $J_{1,2'} = 10.3$ Hz, $J_{1',F5} = 1.3$ Hz, H-1'), 3.94–3.67 (m, 5H, H-2', H-4', H-5', H-6a', H-6b'); ^{13}C NMR (CD_3OD): δ 159.22, 151.38, 139.15, 127.52, 119.28, 88.32, 79.01, 71.93, 71.70, 68.18, 61.37; IR (Nujol, cm^{-1}): 2230 (CN); Anal. Calcd for $C_{11}H_{12}FN_3O_7$: C, 41.65; H, 3.81; N, 13.25. Found: C, 41.94; H, 3.71; N, 13.51; ESI-MS (m/z) 318.20 ($M + H^+$).

4.3.3. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano- β -D-glucopyranosyl)uracil (**5b**)

Uracil derivative **5b** was synthesized from **4** by the similar procedure as described for **5a**. It was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure **5b** (0.17 g, 61%, $R_f = 0.29$ in EtOAc/hexane, 1:1) as a white solid, mp 190–192 °C. $[\alpha]_D^{22} - 7$ (c 0.7, $CHCl_3$); λ_{max} 260 nm (ϵ 5777); 1H NMR ($CDCl_3$): δ 8.34 (br s, 1H, NH), 7.33 (d, 1H, $J_{5,6} = 8.1$ Hz, H-6), 6.09 (d, 1H, $J_{1,2'} = 9.5$ Hz, H-1'), 5.85 (d, 1H, H-5), 5.79 (d, 1H, H-2'), 5.68 (d, 1H, $J_{4',5'} = 10.2$ Hz, H-4'), 4.46–4.42 (m, 1H, H-5'), 4.22–4.17 (m, 2H, H-6a', H-6b'), 2.19, 2.14, 2.12, 2.07 (4s, 12H, 4OAc); Anal. Calcd for $C_{19}H_{21}N_3O_{11}$: C, 48.83; H, 4.53; N, 8.99. Found: C, 48.65; H, 4.11; N, 8.79; ESI-MS (m/z) 468.40 ($M + H^+$).

4.3.4. Synthesis of 1-(3'-C-cyano- β -D-glucopyranosyl)uracil (**6b**)

Uracil derivative **6b** was synthesized from **5b** by the similar procedure as described for **6a**. The residue was purified by flash chromatography (CH_2Cl_2 /MeOH, 9:1) to afford pure **6b** (70 mg, 64%, $R_f = 0.29$ in CH_2Cl_2 /MeOH, 8:2) as a white foam. $[\alpha]_D^{22} + 7$ (c 0.32, CD_3OD); λ_{max} 258 nm (ϵ 11306); 1H NMR (CD_3OD): δ 7.70 (d, 1H, $J_{5,6} = 8.0$ Hz, H-6), 6.01 (d, 1H, $J_{1,2'} = 9.0$ Hz, H-1'), 5.70 (d, 1H, H-5), 3.97–3.54 (m, 5H, H-2', H-4', H-5', H-6a', H-6b'); ^{13}C NMR (CD_3OD): δ 164.12, 150.23, 141.44, 119.01, 102.6, 92.22, 79.68, 72.12, 70.9, 68.35, 61.32; IR (Nujol, cm^{-1}): 2240 (CN); Anal. Calcd for $C_{11}H_{13}N_3O_7$: C, 44.15; H, 4.38; N, 14.04. Found: C, 43.90; H, 4.28; N, 13.96; ESI-MS (m/z) 300.22 ($M + H^+$).

4.3.5. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano- β -D-glucopyranosyl)thymine (**5c**)

Thymine derivative **5c** was synthesized from **4** by the similar procedure as described for **5a**. It was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure **5c** (0.16 g, 57%, $R_f = 0.34$ in EtOAc/hexane, 1:1) as a white solid, mp 127–129 °C. $[\alpha]_D^{22} - 4$ (c 0.39, $CHCl_3$); λ_{max} 259 nm (ϵ 9402); 1H NMR ($CDCl_3$): δ 8.34 (br s, 1H, NH), 7.01 (s, 1H, H-6), 6.08 (d, 1H, $J_{1,2'} = 9.6$ Hz, H-1'), 5.80 (d, 1H, H-2'), 5.67 (d, 1H, $J_{4',5'} = 10.2$ Hz, H-4'), 4.47–4.38 (m, 1H, H-5'), 4.23–4.13 (m, 2H, H-6a', H-6b'), 2.18, 2.14, 2.12, 2.07 (4s, 12H, 4OAc), 1.97 (s, 3H, CH_3); Anal. Calcd for $C_{20}H_{23}N_3O_{11}$: C, 49.90; H, 4.82; N, 8.73. Found: C, 49.62; H, 4.58; N, 8.71; ESI-MS (m/z) 482.42 ($M + H^+$).

Table 1

Cytostatic activity of **6a–d** and **12a–d** against a panel of tumor cell lines.

Compound	IC ₅₀ ^a (μ M)		
	L1210	CEM	HeLa
6a	1.9 \pm 0.0	32 \pm 9.6	4.5 \pm 1.2
6b	417 \pm 376	> 500	> 500
6c	539 \pm 85	> 500	> 500
6d	147 \pm 14	> 500	377 \pm 0
12a	550 \pm 10	> 500	819 \pm 44
12b	> 500	> 500	811 \pm 40
12c	> 750	> 750	> 750
12d	> 750	> 750	> 750
F-Uracil	0.49 \pm 0.13	18 \pm 5	0.54 \pm 0.12

^a 50% inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%. Data are the mean of 2–3 independent experiments (\pm S.D.).

4.3.6. Synthesis of 1-(3'-C-cyano-β-D-glucopyranosyl)thymine (**6c**)

Thymine derivative **6c** was synthesized from **5c** by the similar procedure as described for **6a**. The residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to afford pure **6c** (114 mg, 71%, Rf = 0.32 in CH₂Cl₂/MeOH, 8:2) as a white foam. $[\alpha]_D^{22} + 4$ (c 0.31, CD₃OD); λ_{\max} 261 nm (ϵ 4238); ¹H NMR (CD₃OD): δ 7.57 (s, 1H, H-6), 6.03 (d, 1H, $J_{1',2'} = 9.0$ Hz, H-1'), 4.00–3.67 (m, 5H, H-2', H-4', H-5', H-6a', H-6b'), 1.91 (s, 3H, CH₃); ¹³C NMR (CD₃OD): δ 162.62, 150.49, 137.12, 119.91, 110.69, 92.76, 79.83, 71.01, 70.90, 69.81, 62.46, 13.33; IR (Nujol, cm⁻¹): 2245 (CN); Anal. Calcd for C₁₂H₁₅N₃O₇: C, 46.01; H, 4.83; N, 13.41. Found: C, 45.79; H, 4.61; N, 13.26; ESI-MS (m/z) 314.28 (M + H⁺).

4.3.7. Synthesis of 1-(2',3',4',6'-tetra-O-acetyl-3'-C-cyano-β-D-glucopyranosyl)N⁴-benzoylcytosine (**5d**)

N⁴-benzoyl cytosine derivative **5d** was synthesized from **4** by the similar procedure as described for **5a**. It was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure **5d** (0.19 g, 56%, Rf = 0.32 in EtOAc/hexane, 1:1) as a white solid, mp 240–242 °C. $[\alpha]_D^{22} - 5$ (c 0.43, CHCl₃); λ_{\max} 263 nm (ϵ 25940); ¹H NMR (CDCl₃): δ 7.95–7.49 (m, 7H, Bz, H-5 and H-6), 6.37 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1'), 5.82 (d, 1H, H-2'), 5.67 (d, 1H, $J_{4',5'} = 10.0$ Hz, H-4'), 4.48–4.40 (m, 1H, H-5'), 4.28–4.14 (m, 2H, H-6a', H-6b'), 2.18, 2.14, 2.11, 2.05 (4s, 12H, 4OAc); Anal. Calcd for C₂₆H₂₆N₄O₁₁: C, 54.74; H, 4.59; N, 9.82. Found: C, 54.61; H, 4.65; N, 9.71; ESI-MS (m/z) 571.48 (M + H⁺).

4.3.8. Synthesis of 1-(3'-C-cyano-β-D-glucopyranosyl)cytosine (**6d**)

Cytosine derivative **6d** was synthesized from **5d** by the similar procedure as described for **6a**. The residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to afford pure **6d** (61 mg, 61%, Rf = 0.23 in CH₂Cl₂/MeOH, 7:3) as a white foam. $[\alpha]_D^{22} - 3$ (c 0.31, CD₃OD); λ_{\max} 269 nm (ϵ 7569); ¹H NMR (CD₃OD): δ 7.70 (d, 1H, $J_{5,6} = 7.5$ Hz, H-6), 6.12 (d, 1H, $J_{1',2'} = 9.0$ Hz, H-1'), 5.94 (d, 1H, H-5), 4.00–3.69 (m, 5H, H-2', H-4', H-5', H-6a', H-6b'); ¹³C NMR (CD₃OD): δ 165.72, 156.91, 143.22, 119.16, 94.96, 91.37, 79.96, 72.01, 70.28, 68.16, 61.92; IR (Nujol, cm⁻¹): 2235 (CN); Anal. Calcd for C₁₁H₁₄N₄O₆: C, 44.30; H, 4.73; N, 18.79. Found: C, 44.48; H, 4.58; N, 18.96; ESI-MS (m/z) 299.23 (M + H⁺).

4.4. Synthesis of 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranose (**10**)

4.4.1. Synthesis of 3-C-cyano-3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**8**)

Phenyl chlorothionoformate (1.39 mL, 10.33 mmol) was added to a solution of **2** (2 g, 7.01 mmol), DMAP (3.03 mmol, 0.37 g), and Et₃N (1.47 mL, 10.69 mmol) in CH₃CN (73.68 mL) under nitrogen at 0 °C. The mixture was stirred for 1 h and then diluted with AcOEt (500 mL). The whole was washed with H₂O (3 × 150 mL) and the separated organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue **7** was coevaporated two times with toluene and then was dissolved in toluene (73.68 mL). Bu₃SnH (2.93 mL, 11.06 mmol) was added to the above solution containing AIBN (1.1 mmol, 182 mg) at 100 °C under nitrogen. After being heated for 45 min, the solvent was removed under reduced pressure. The residue was purified by flash chromatography (EtOAc/hexane, 3:7) to give compound **8** (1.43 g, 76%, Rf = 0.44 in EtOAc/hexane, 3:7) as a white solid: mp 104–106 °C. $[\alpha]_D^{22} + 16$ (c 0.40, CHCl₃); ¹H NMR (CDCl₃): δ 5.81 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.76 (dd, 1H, $J_{2,3} = 4.8$ Hz, H-2), 4.21–3.94 (m, 4H, H-4, H-5, H-6a, H-6b), 2.88 (dd, 1H, H-3), 1.51 (s, 3H, CH₃), 1.42 (s, 3H, CH₃), 1.33 (s, 6H, 2 CH₃). Anal. Calcd for C₁₃H₁₉N₃O₅: C, 57.98; H, 7.11; N, 5.20. Found: C, 58.12; H, 7.20; N, 5.41; ESI-MS (m/z) 270.27 (M + H⁺).

4.4.2. Synthesis of 3-C-cyano-3-deoxy-α-D-allopyranose (**9**)

To a solution of **8** (1.43 g, 5.31 mmol) in MeOH (10.9 mL) and H₂O (62.2 mL) was added Amberlite IR 120 (H⁺) resin and the mixture was refluxed overnight. The reaction mixture was filtered and evaporated to dryness to give compound **9** (924 mg, 92%, Rf = 0.17 in EtOAc) as a viscous oil, and it was used without further purification. $[\alpha]_D^{22} + 6$ (c 0.45, MeOH); Anal. Calcd for C₇H₁₁N₃O₅: C, 44.45; H, 5.86; N, 7.40. Found: C, 44.21; H, 5.92; N, 7.22; ESI-MS: (m/z) 190.20 (M + H⁺).

4.4.3. Synthesis of 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranose (**10**)

Compound **9** (924 mg, 4.89 mmol) was dissolved in a mixture of pyridine (17.12 mL) and Ac₂O (8.8 mL). The reaction was carried out at room temperature for 1 h, then was quenched with MeOH at 0 °C and was concentrated in vacuum. The residue was diluted with EtOAc, washed with saturated sodium bisulfate, sodium bicarbonate and H₂O. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give compound **10** (1.56 g, 89%, Rf = 0.41 in EtOAc/hexane, 3:7) as a colorless oil. $[\alpha]_D^{22} - 2$ (c 0.55, CHCl₃); Anal. Calcd for C₁₅H₁₉N₃O₉: C, 50.42; H, 5.36; N, 3.92. Found: C, 50.54; H, 5.32; N, 3.87; ESI-MS: (m/z) 358.30 (M + H⁺).

4.5. Synthesis of 3'-C-cyano-3'-deoxy-β-D-allopyranonucleosides **12a–d**

4.5.1. Synthesis of 1-(2',4',6'-tri-O-acetyl-3'-C-cyano-3'-deoxy-β-D-allopyranosyl)5-fluorouracil (**11a**)

A mixture of 5-fluorouracil (126 mg, 0.97 mmol), HMDS (254 μL, 1.20 mmol) and saccharine (8 mg, 0.045 mmol) in anhydrous CH₃CN (3.5 mL) was refluxed for 30 min under nitrogen. 1,2,4,6-tetra-O-acetyl-3-C-cyano-3-deoxy-D-allopyranose (**10**) (0.25 g, 0.69 mmol) and Me₃SiOSO₂CF₃ (152 μL, 0.84 mmol) were then added and the reaction mixture was refluxed for 4 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH₂Cl₂ (200 mL). The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 4:6) to give pure **11a** (0.19 g, 64%, Rf = 0.33 in EtOAc/hexane, 1:1) as a white solid, mp 108–110 °C. $[\alpha]_D^{22} - 2$ (c 0.28, CHCl₃); λ_{\max} (CHCl₃) 263 nm (ϵ 9073); ¹H NMR (CDCl₃): δ 8.58 (br s, 1H, NH), 7.18 (d, 1H, $J_{6,F5} = 5.6$ Hz, H-6), 5.93 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1'), 4.92 (dd, 1H, $J_{2',3'} = 5.0$ Hz, H-2'), 5.79 (dd, 1H, $J_{3',4'} = 4.9$ Hz, $J_{4',5'} = 9.7$ Hz, H-4'), 4.21–4.06 (m, 3H, H-6a', H-6b', H-5'), 3.89 (t, 1H, H-3'), 2.03, 1.98, 1.96, (3s, 9H, 3OAc); Anal. Calcd for C₁₇H₁₈FN₃O₉: C, 47.78; H, 4.25; N, 9.83. Found: C, 47.56; H, 4.31; N, 9.79; ESI-MS (m/z) 428.36 (M + H⁺).

4.5.2. Synthesis of 1-(3'-C-cyano-3'-deoxy-β-D-allopyranosyl)5-fluorouracil (**12a**)

To a solution of compound **11a** (0.19 g, 0.44 mmol) in AcOH-pyridine (2.2 mL, 1:4 v/v), 85% hydrazine hydrate (0.31 mL, 5.28 mmol) was added at room temperature. After continually stirring for 16h, acetone (1.1 mL) was added, and stirring for an additional 30 min. The mixture was evaporated to dryness and the residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to afford pure **12a** (86 mg, 65%, Rf = 0.26 in CH₂Cl₂/MeOH, 9:1) as a white foam. $[\alpha]_D^{22} - 8$ (c 0.65, CD₃OD); λ_{\max} 263 nm (ϵ 8929); ¹H NMR (CD₃OD): δ 7.80 (d, 1H, $J_{6,F5} = 6.5$ Hz, H-6), 6.01 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1'), 4.46–4.42 (m, 1H, H-5'), 4.23 (dd, 1H, $J_{3',4'} = 5.6$ Hz, $J_{4',5'} = 12.1$ Hz, H-4'), 4.05 (dd, 1H, $J_{2',3'} = 5.1$ Hz, H-2'), 3.95–3.84 (m, 2H, H-6a', H-6b'), 3.75 (t, 1H, H-3'); ¹³C NMR (CD₃OD): δ 158.09, 150.72, 141.39, 128.03, 118.43, 96.49, 84.37, 67.33, 64.11, 61.09, 29.12; IR (Nujol, cm⁻¹): 2250 (CN); Anal. Calcd for C₁₁H₁₂FN₃O₆: C, 43.86; H, 4.02; N, 13.95. Found: C, 43.74; H, 3.94; N, 13.82; ESI-MS (m/z) 302.21 (M + H⁺).

4.5.3. Synthesis of 1-(2',4',6'-tri-O-acetyl-3'-C-cyano-3'-deoxy-β-D-allopyranosyl)uracil (**11b**)

Uracil derivative **11b** was synthesized from **10** by the similar procedure as described for **11a**. It was purified by flash chromatography (EtOAc/hexane, 1:1) to give pure **11b** (0.18 g, 62%, Rf = 0.2 in EtOAc/hexane, 1:1) as a white solid, mp 124–126 °C. $[\alpha]_D^{22} - 2$ (c 0.30, CHCl₃); λ_{\max} 256 nm (ϵ 13439); ¹H NMR (CDCl₃): δ 8.27 (br s, 1H, NH), 7.25 (d, 1H, J_{5,6} = 8.2 Hz, H-6), 6.11 (d, 1H, J_{1',2'} = 9.6 Hz, H-1'), 5.80 (d, 1H, H-5), 5.12 (dd, 1H, J_{2',3'} = 5.2 Hz, H-2'), 4.96 (dd, 1H, J_{3',4'} = 5.0 Hz, J_{4',5'} = 9.6 Hz, H-4'), 4.38–4.23 (m, 3H, H-6a', H-6b', H-5'), 4.06 (t, 1H, H-3'), 2.20, 2.15, 2.12 (s, 9H, 3OAc); Anal. Calcd for C₁₇H₁₉N₃O₉: C, 49.88; H, 4.68; N, 10.27. Found: C, 49.65; H, 4.51; N, 10.36; ESI-MS (*m/z*) 410.32 (M + H⁺).

4.5.4. Synthesis of 1-(3'-C-cyano-3'-deoxy-β-D-allopyranosyl)uracil (**12b**)

Uracil derivative **12b** was synthesized from **11b** by the similar procedure as described for **12a**. The residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to afford pure **12b** (91 mg, 75%, Rf = 0.19 in CH₂Cl₂/MeOH, 9:1) as a white foam. $[\alpha]_D^{22} - 22$ (c 0.80, CD₃OD); λ_{\max} 261 nm (ϵ 15441); ¹H NMR (CD₃OD): δ 7.61 (d, 1H, J_{5,6} = 8.1 Hz, H-6), 5.82 (d, 1H, J_{1',2'} = 9.3 Hz, H-1'), 5.72 (d, 1H, H-5), 4.46–4.42 (m, 1H, H-5'), 4.23 (dd, 1H, J_{3',4'} = 5.4 Hz, J_{4',5'} = 12.1 Hz, H-4'), 4.08 (dd, 1H, J_{2',3'} = 5.1 Hz, H-2'), 3.99–3.84 (m, 2H, H-6a', H-6b'), 3.76 (t, 1H, H-3'); ¹³C NMR (CD₃OD): δ 164.01, 150.12, 142.27, 118.96, 102.13, 98.17, 83.96, 66.89, 63.01, 61.20, 30.87; IR (Nujol, cm⁻¹): 2245 (CN); Anal. Calcd for C₁₁H₁₃N₃O₆: C, 46.65; H, 4.63; N, 14.84. Found: C, 46.96; H, 4.78; N, 14.75; ESI-MS (*m/z*) 284.22 (M + H⁺).

4.5.5. Synthesis of 1-(2',4',6'-tri-O-acetyl-3'-C-cyano-3'-deoxy-β-D-allopyranosyl)thymine (**11c**)

Thymine derivative **11c** was synthesized from **10** by the similar procedure as described for **11a**. It was purified by flash chromatography (EtOAc/hexane, 1:1) to give pure **11c** (0.19 g, 68%, Rf = 0.25 in EtOAc/hexane, 1:1) as a white solid, mp 106–108 °C. $[\alpha]_D^{22} - 4$ (c 0.30, CHCl₃); λ_{\max} (CHCl₃) 261 nm (ϵ 7290); ¹H NMR (CDCl₃): δ 8.26 (br s, 1H, NH), 7.04 (s, 1H, H-6), 6.1 (d, 1H, J_{1',2'} = 9.7 Hz, H-1'), 5.12 (dd, 1H, J_{2',3'} = 5.2 Hz, H-2'), 5.67 (dd, 1H, J_{3',4'} = 4.9 Hz, J_{4',5'} = 9.6 Hz, H-4'), 4.38–4.23 (m, 3H, H-6a', H-6b', H-5'), 4.06 (t, 1H, H-3'), 2.19, 2.13, 2.12 (3s, 9H, 3OAc), 1.96 (s, 3H, 5-CH₃); Anal. Calcd for C₁₈H₂₁N₃O₉: C, 51.06; H, 5.00; N, 9.93. Found: C, 50.87; H, 4.96; N, 9.87; ESI-MS (*m/z*) 424.39 (M + H⁺).

4.5.6. Synthesis of 1-(3'-C-cyano-3'-deoxy-β-D-allopyranosyl)thymine (**12c**)

Thymine derivative **12c** was synthesized from **11c** by the similar procedure as described for **12a**. The residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to afford pure **12c** (99 mg, 71%, Rf = 0.20 in CH₂Cl₂/MeOH, 9:1) as a white foam. $[\alpha]_D^{22} - 16$ (c 0.50, CD₃OD); λ_{\max} 262 nm (ϵ 14609); ¹H NMR (CD₃OD): δ 7.40 (s, 1H, H-6), 5.77 (d, 1H, J_{1',2'} = 9.4 Hz, H-1'), 4.46–4.42 (m, 1H, H-5'), 4.24 (dd, 1H, J_{3',4'} = 5.6 Hz, J_{4',5'} = 12.0 Hz, H-4'), 4.11 (dd, 1H, J_{2',3'} = 5.1 Hz, H-2'), 3.99–3.84 (m, 2H, H-6a', H-6b'), 3.70 (t, 1H, H-3'), 1.91 (s, 3H, 5-CH₃); ¹³C NMR (CD₃OD): δ 163.67, 150.19, 136.85, 119.01, 110.63, 97.67, 84.15, 67.29, 64.55, 61.30, 28.02, 12.68; IR (Nujol, cm⁻¹): 2240 (CN); Anal. Calcd for C₁₂H₁₅N₃O₆: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.82; H, 5.12; N, 14.09; ESI-MS (*m/z*) 298.27 (M + H⁺).

4.5.7. Synthesis of 1-(2',4',6'-tri-O-acetyl-3'-C-cyano-3'-deoxy-β-D-allopyranosyl)N⁴-benzoylcytosine (**11d**)

N⁴-benzoylcytosine derivative **11d** was synthesized from **10** by the similar procedure as described for **11a**. It was purified by flash

chromatography (EtOAc/hexane, 1:1) to give pure **11d** (0.21 g, 59%, Rf = 0.23 in EtOAc/hexane, 1:1) as a white solid, mp 203–205 °C. $[\alpha]_D^{22} + 2$ (c 0.28, CHCl₃); λ_{\max} 264 nm (ϵ 14496); ¹H NMR (CDCl₃): δ 7.94–7.51 (m, 7H, Bz, H5 and H-6), 6.40 (d, 1H, J_{1',2'} = 9.6 Hz, H-1'), 5.17 (dd, 1H, J_{2',3'} = 5.2 Hz, H-2'), 5.01 (dd, 1H, J_{3',4'} = 5.0 Hz, J_{4',5'} = 9.9 Hz, H-4'), 4.41–4.24 (m, 3H, H-6a', H-6b', H-5'), 4.06 (t, 1H, H-3'), 2.19, 2.14, 2.11 (3s, 9H, 3OAc); Anal. Calcd for C₂₄H₂₄N₄O₉: C, 56.25; H, 4.72; N, 10.93. Found: C, 56.64; H, 4.69; N, 10.68; ESI-MS (*m/z*) 513.48 (M + H⁺).

4.5.8. Synthesis of 1-(3'-C-cyano-3'-deoxy-β-D-allopyranosyl)cytosine (**12d**)

Cytosine derivative **12d** was synthesized from **11d** by the similar procedure as described for **12a**. The residue was purified by flash chromatography (CH₂Cl₂/MeOH, 9:1) to afford pure **12d** (33 mg, 55%, Rf = 0.25 in CH₂Cl₂/MeOH, 7:3) as an orange foam. $[\alpha]_D^{22} - 6$ (c 0.50, CD₃OD); λ_{\max} 266 nm (ϵ 12306); ¹H NMR (CD₃OD): δ 7.68 (d, 1H, J_{5,6} = 7.5 Hz, H-6), 5.97 (d, 1H, J_{1',2'} = 9.0 Hz, H-1'), 5.97 (d, 1H, H-5), 4.13–3.74 (m, 6H, H-2', H-3', H-4', H-5', H-6a', H-6b'); ¹³C NMR (CD₃OD): δ 165.79, 155.73, 143.46, 118.86, 96.37, 94.96, 84.01, 67.38, 63.71, 61.32, 27.83; IR (Nujol, cm⁻¹): 2235 (CN); Anal. Calcd for C₁₁H₁₄N₄O₅: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.56; H, 4.78; N, 19.41; ESI-MS (*m/z*) 283.28 (M + H⁺).

4.5.9. Antiviral and cytostatic assays

The antiviral assays [except anti-human immunodeficiency virus (HIV) assays] were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus], Vero (para-influenza-3, reovirus-1, Cocksackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Cocksackie virus B4, and respiratory syncytial virus), MDCK (influenza A (H1N1; H3N2) and B virus) and CrFK (feline corona virus (FIPV) and feline herpes virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 (CCID₅₀) of virus (1 CCID₅₀ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations (5,000, 1,000, 200 ... nM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

The anti-HIV activity and anti-proliferative activity were evaluated against HIV-1 strain IIIB and HIV-2 strain ROD in human T-lymphocyte CEM cell cultures. Briefly, virus stocks were titrated in CEM cells and expressed as the 50% cell culture infective dose (CCID₅₀). CEM cells were suspended in culture medium at $\sim 3 \times 10^5$ cells/ml and infected with HIV at ~ 100 CCID₅₀. Immediately after viral exposure, 100 μ l of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4-day incubation period at 37 °C, the giant cell formation was microscopically determined. Compounds were tested in parallel for cytostatic effects in uninfected CEM cells.

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References

- [1] W. Zhou, G. Gumina, Y. Chong, J. Wang, R.F. Shinazi, C.K. Chu, *J. Med. Chem.* 47 (2004) 3399–3408.
- [2] R.K. Robins, G.D. Kini, in: D.E.V. Wilman (Ed.), *The Chemistry of Antitumor Agents*, Chapman and Hall, New York, 1990, pp. 299–321.
- [3] M. MacCoss, M.J. Robins, in: D.E.V. Wilman (Ed.), *The Chemistry of Antitumor Agents*, Chapman and Hall, New York, 1990, pp. 261–298.
- [4] D. Komiotis, S. Manta, E. Tsoukala, N. Tzioumaki, *Curr. Med. Chem. Anti-Infect. Agents* 7 (2008) 219–244.
- [5] C.M. Galmarini, J.R. Mackey, C. Dumontet, *Lancet Oncol.* 3 (2002) 415–424.
- [6] E. De Clercq, *Biochim. Biophys. Acta* 1587 (2002) 258–275.
- [7] R.C. Brady, D.I. Bernstein, *Antivir. Res.* 61 (2004) 73–81.
- [8] K. Takenuki, A. Matsuda, T. Ueda, T. Sasaki, A. Fujii, K. Yamagami, *J. Med. Chem.* 31 (1988) 1063–1064.
- [9] J.R. McCarthy, D.P. Matthews, D.M. Stemerick, E.W. Huber, P. Bey, B.J. Lippert, R.D. Snyder, P.S. Sunkara, *J. Am. Chem. Soc.* 113 (1991) 7439–7440.
- [10] H. Hattori, M. Tanaka, M. Fukushima, T. Sasaki, A. Matsuda, *J. Med. Chem.* 39 (1996) 5005–5011.
- [11] H. Hayakawa, S. Kohgo, K. Kitano, N. Ashida, E. Kodama, H. Mitsuya, H. Ohrai, *Antiviral. Chem. Chemother.* 15 (2004) 169–187.
- [12] M.J. Camarasa, A. Diaz-Ortiz, A. Calvo-Mateo, F.G. De las Heras, J. Balzarini, E. De Clercq, *J. Med. Chem.* 32 (1989) 1732–1738.
- [13] C. O-Yang, H.Y. Wu, E.B. Fraser-Smith, K.A.M. Walker, *Tetrahedron Lett.* 33 (1992) 37–40.
- [14] W. Zhu, G. Gumina, R.F. Schinazi, C.K. Chu, *Tetrahedron* 59 (2003) 6423–6431.
- [15] A. Matsuda, Y. Nakajima, A. Azuma, M. Tanaka, T. Sasaki, *J. Med. Chem.* 34 (1991) 2917–2919.
- [16] A. Azuma, Y. Nakajima, N. Nishizono, N. Minakawa, M. Suzuki, K. Hanaoka, T. Kobayashi, M. Tanaka, T. Sasaki, A. Matsuda, *J. Med. Chem.* 36 (1993) 4183–4189.
- [17] A. Azuma, P. Huang, A. Matsuda, W. Plunkett, *Mol. Pharmacol.* 59 (2001) 725–731.
- [18] I. Verheggen, A. Van Aerschot, L. Van Meervelt, J. Rozenski, L. Wiebe, R. Snoeck, G. Andrei, J. Balzarini, P. Claes, E. De Clercq, P. Herdewijn, *J. Med. Chem.* 38 (1995) 826–835.
- [19] T. Ostrowski, B. Wroblewski, R. Busson, J. Rozenski, E. De Clercq, M.S. Bennet, J.N. Champness, W.C. Summers, M.R. Sanderson, P. Herdewijn, *J. Med. Chem.* 41 (1998) 4343–4353.
- [20] Y. Maurinsh, J. Schraml, H. De Winter, N. Blaton, O. Peeters, E. Lesclerq, J. Rozenski, A. Van Aerschot, E. De Clercq, R. Busson, P. Herdewijn, *J. Org. Chem.* 62 (1997) 2861–2871.
- [21] C. Spanou, S. Manta, D. Komiotis, A. Dervishi, D. Kouretas, *Int. J. Mol. Sci.* 8 (2007) 695–704.
- [22] A. Haouz, V. Vanheusden, H. Munier-Lechman, M. Froeyen, P. Herdewijn, S. Van Calenbergh, M. Delarue, *J. Biol. Chem.* 278 (2003) 4963–4971.
- [23] K. Vastmans, S. Pochet, A. Peys, L. Kerremans, A. Van Aerschot, C. Hendrix, P. Marliere, P. Herdewijn, *Biochemistry* 39 (2000) 12757–12765.
- [24] K. Vastmans, M. Froeyen, L. Kerremans, S. Pochet, P. Herdewijn, *Nucleic Acids Res.* 29 (2001) 3154–3163.
- [25] S. Manta, G. Agelis, T. Botić, A. Cencić, D. Komiotis, *Bioorg. Med. Chem.* 15 (2007) 980–987.
- [26] S. Manta, G. Agelis, T. Botić, A. Cencić, D. Komiotis, *Eur. J. Med. Chem.* 43 (2008) 420–428.
- [27] S. Manta, E. Tsoukala, N. Tzioumaki, A. Goropevsek, R.T. Pamulapati, A. Cencić, D. Komiotis, *Eur. J. Med. Chem.* 44 (2009) 2696–2704.
- [28] S. Manta, N. Tzioumaki, E. Tsoukala, A. Panagiotopoulou, M. Pelecanou, J. Balzarini, D. Komiotis, *Eur. J. Med. Chem.* 44 (2009) 4764–4771.
- [29] E. Tsoukala, N. Tzioumaki, S. Manta, A. Riga, J. Balzarini, D. Komiotis, *Bioorg. Chem.* 38 (2010) 285–293.
- [30] N.A.A. Balatsos, D. Vlachakis, P. Maragozidis, S. Manta, D. Anastakis, A. Kyritsis, M. Vlassi, D. Komiotis, C. Stathopoulos, *Biochemistry* 48 (2009) 6044–6051.
- [31] V.G. Tsirkone, E. Tsoukala, C. Lamprakis, S. Manta, J.M. Hayes, V.T. Skamnaki, C. Drakou, S.E. Zographos, D. Komiotis, D.D. Leonidas, *Bioorg. Med. Chem.* 18 (2010) 3423–3425.
- [32] J. Elhalabi, K.G. Rice, *Nucleosides Nucleotides Nucleic Acids* 23 (2004) 195–205.
- [33] J.M. Bourgeois, *Helv. Chim. Acta* 58 (1975) 363–372.
- [34] J.A. Rosenthal, B.L. Cliff, *Can. J. Chem.* 54 (1976) 543–547.
- [35] H. Vorbruggen, G. Hofle, *Chem. Ber* 114 (1981) 1256–1268.
- [36] V. Vanheusden, R. Busson, P. Herdewijn, S. Van Calenbergh, *J. Org. Chem.* 69 (2004) 4446–4453.